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carbohydratesisomersketone starchlipidproteinamine

# Biochemistry 2

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

**Done By**

Adnan Al-Kababji & Abod Suliman

**Contributed in the Scientific Correction**

Adnan Al-Kababji & Abod Suliman

**Contributed in the Grammatical Correction**

Saif Shteiwi

**Doctor**

Faisal Al-Khatieb

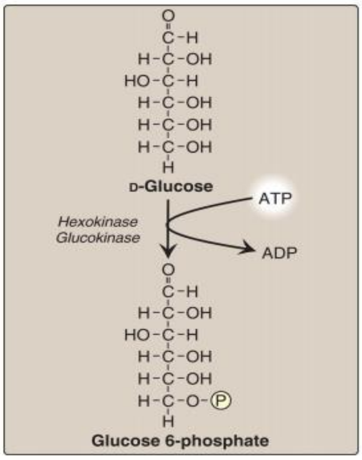
# Glycolysis Overview

- The glycolytic pathway is used by all tissues for the oxidation of Glucose to proivde energy in the form of ATP, and to provide intermediates for other metabolic pathways.
- The intermediates in this pathway are either 6 carbons (glucose 6-P, fructose 6-P,…) or 3 carbons (glyceraldehyde 3-P,…)

## ❖ Glycolysis Reaction

- The Glycolysis reaction consists of 10 reactions, the first five reactions correspond to an **energy-investment phase (preparative phase),** and the subsequent reactions constitute an **energy-generation phase.**
- Glycolysis (aerobic) produces **2 ATP**, **2 NADH,** and **2 Pyruvate molecules.**

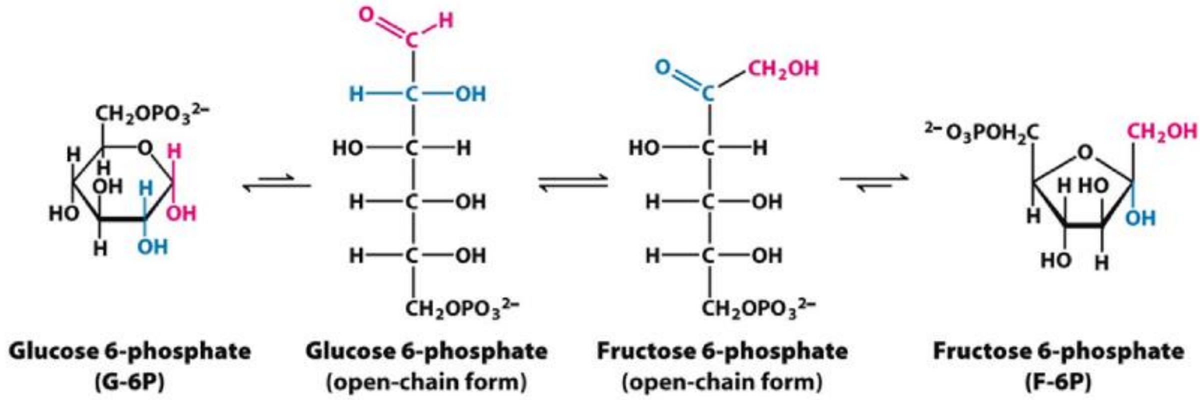
1. **Glucose phosphorylation (first rate-limiting step)**




- The enzymes that catalyze this reaction are **Hexokinase, Glucokinase**
- This reaction is **the first irreversible reaction** since a high energy bond in ATP is broken, and a low energy bond (ester bond) is formed in Glucose 6-P. This is considered as an exergonic reaction.
- Since the reaction is exergonic and irreversible, it should be **regulated**.
- Phosphorylated sugar molecules do not penetrate cell membrane (no longer able to leave the cell ''trapped'') since:
  - ✓ there are no specific transmembrane carriers for these compounds
  - ✓ they are too polar (Phosphate group is highly negative charged).

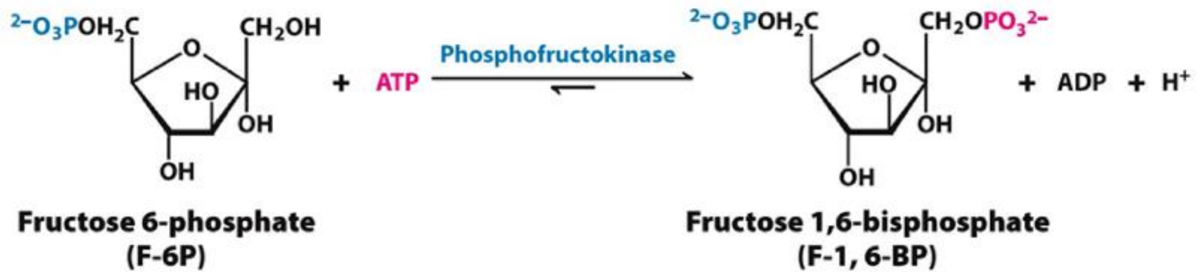
- **as a result,** Phosphate group will be trapped also, because adding Phosphate group will decrease the level of free Phosphates which is required for ATP synthesis (ATP required ADP+Pi).

2. **Glucose 6-P isomerization**



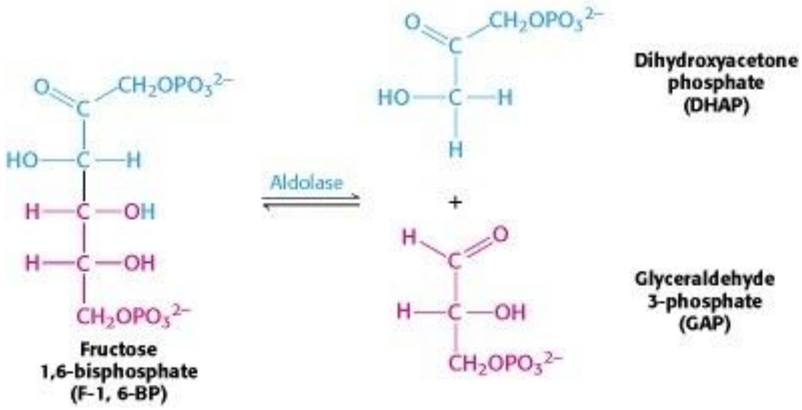

- The enzyme that catalyzes this reaction is **Phosphoglucose isomerase**.
- The reaction is freely reversible and doesn't require regulation.
- The isomerization from a 6 membered ring (G-6P) to 5 membered ring (F-6P) is done through the open-chain form.

3. **Fructose 6-phosphate phosphorylation (the committed step)**



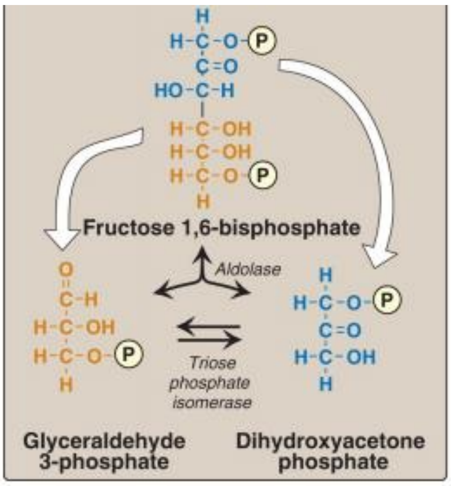

- The enzyme that catalyzes this reaction is **Phosphofructokinase-1 (PFK-1)**.
- In this reaction another Phosphate group is added to C1 of F-6P.
- This reaction is **the second irreversible reaction.** Therefore, it is highly regulated, [note: The conversion from F-6P to F-1,6-BP cannot be done spontaneously through the enzyme since it's an endergonic reaction. However, coupling the reaction with ATP produces and overall **exergonic reaction**, thus making it possible for the enzyme. (**large - ΔG**)].
- This reaction is a **rate-limiting** and **committed step** because F-1,6-BP is only used in Glycolysis unlike G-6P which can be used in more than one reaction.

## 4. Fructose 1,6-bisphosphate cleavage



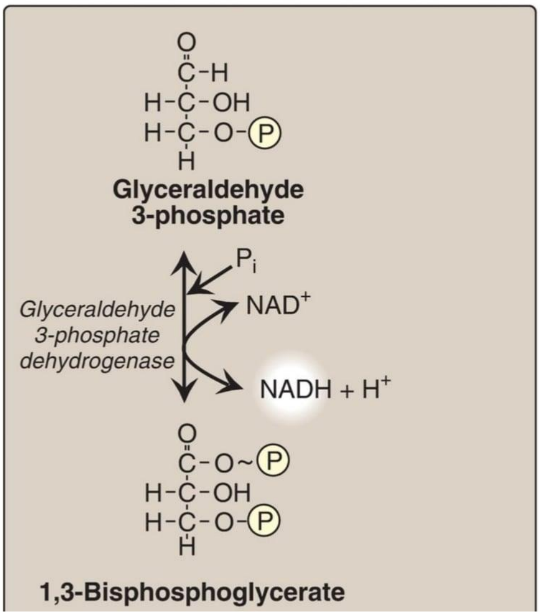

- The cleavage of **F-1,6-BP** into **Glyceraldehyde 3-Phosphate (GAP)** and **Dihydroxyacetone Phosphate (DHAP)** is catalyzed by an enzyme called **Aldolase**.
- The cleavage occurs between C3 and C4.
- The cleavage does not require water since it's a lyase reaction not hydrolyses.
- This reaction is reversible and not regulated.

## 5. Dihydroxyacetone phosphate isomerization



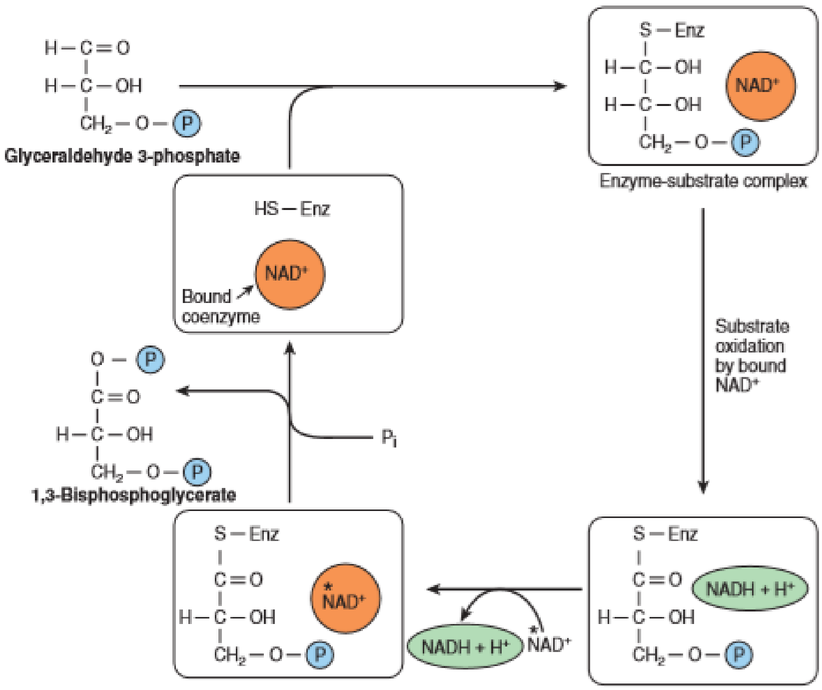

- The enzyme that catalyzes this reaction is **Triose phosphate isomerase,** which interconverts **DHAP** and **GAP.**
- **DHAP** must be isomerized to **GAP** for further metabolism by glycolytic pathway.
- This isomerization results in the net production of **2 molecules of GAP.**

### 6. Glyceraldehyde 3-phosphate oxidation (the first oxidation-reduction reaction)




- The enzyme that catalyzes this reaction is **Glyceraldehyde 3-phosphate dehydrogenase (GAP dehydrogenase).**
- GAP dehydrogenase converts **GAP** to **1,3-bisphophoglycerate (1,3-BPG).**
- **A phosphate group** is added to **GAP** to produce **1,3-BPG**. This phosphate group linked to **C1** of the **1,3-BPG** by a high energy bond, conserves much of the free energy produced by the oxidation of GAP.
- In this step, **1 (NADH)** is produced **per 1 (GAP)**, So, for each glucose (2 GAP), **the net production is 2 NADH.**

➢ **How does this reaction occur?**




Mechanisms of action:
E and S form covalent linkage

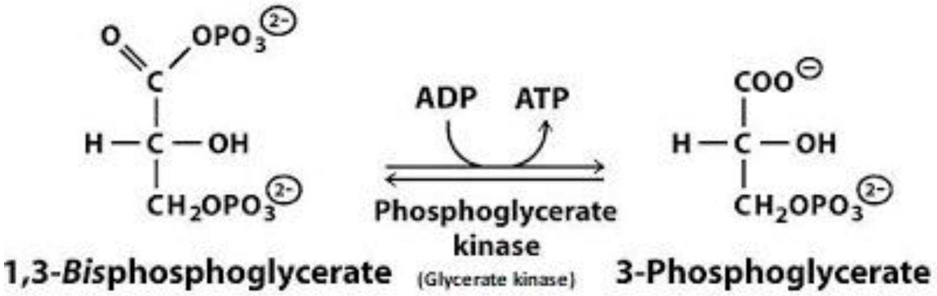
S is oxidized and NADH is formed NADH is released

Pi attacks the thioester bond releasing the product

- ✓ The enzyme has **cysteine** at its side chain which make a covalent bond with the substrate producing **thiohemiacetal** **(organic functional group with the general formula RCH(OH)SR).**
- ✓ **Thiohemiacetal** is oxidized and loses two hydrogen atoms to form an **acyl thioester intermediate**, which is highly energy bound (due to the thioester bond), and **$NAD^+$** is reduced to **NADH** (the compound is still bound to the enzyme).
- ✓ **Now** a **phosphate group** attacks the enzyme-substrate complex (thioester bond), which leads to release of **the product** (**1,3-BPG**) and **the free enzyme** (the released energy from thioester bond is used to bind the **Phosphate group**).

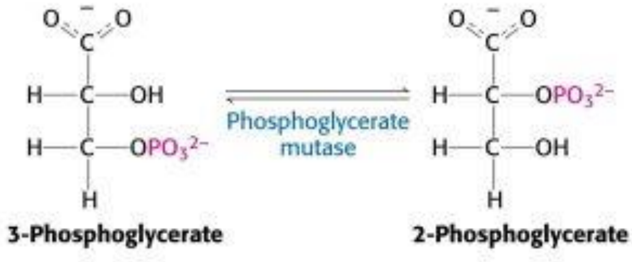
**Note:** If **H2O** attacks the thioester bond (abnormal), **3-Phosphoglycerate (3-PG)** is produced and the energy is released in the form of **heat.** This leads to the decrease in the amount of ATP produced by this pathway, because in this reaction, the step that produces ATP is **bypassed** (will be discussed later).

## 7. 3-Phosphoglycerate synthesis and ATP production



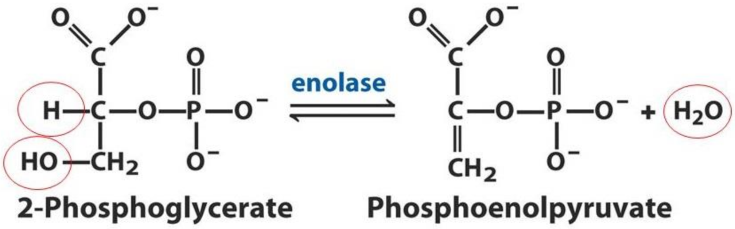

- The enzyme that catalyzes this reaction is **Phosphoglycerate kinase (Glycerate kinase).**
- It's a reversible reaction and has a large negative ΔG.
- In this reaction **1,3-BPG** is converted to **3-Phosphoglycerate (3-PG)**, **the high energy Phosphate group** of 1,3-BPG (at C1) is used to synthesize **ATP from ADP**.
- This Kinase, unlike most kinases, **is physiologically reversible**; which means that **it phosphorylates ADP to ATP**.
- This reaction is an example of **substrate level phosphorylation**, in which the energy needed to produce high energy Phosphate comes from a substrate rather than from **ETC** (Electron Transport Chain).
- In this step **1 (ATP)** is produced **per 1 (1,3-BPG)**, so for each glucose molecule (2 of 1,3-BPG), **the net production is 2 ATP.**

8. **Phosphate group Shift**



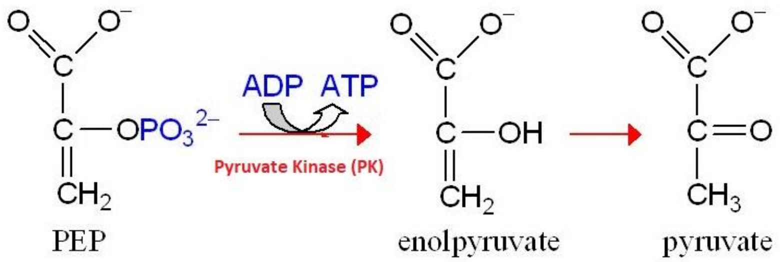

- The enzyme that catalyzes this reaction is **Phosphoglycerate mutase,** which **shifts** the Phosphate group from C3 to C2.
- So, 3-Phosphoglycerate (3-PG) is **isomerized** into 2-phosphoglycerate (2-PG).
- It's a freely reversible reaction.

9. **2-Phoshoglycerate dehydration**




- The enzyme that catalyzes this dehydration reaction is **Enolase (phosphopyruvate hydratase).** It dehydrates 2-Phosphoglycerate (2-PG) by removing water (OH from C3 and H from C2) (enolase redistributes the energy).
- Removing water produces a compound with a double bond called **Phosphoenolpyruvate (PEP).**
- The product (PEP) contains a high energy enol Phosphate.
- This reaction is reversible **despite** the high energy nature of the product.

10. **Pyruvate synthesis and ATP production**



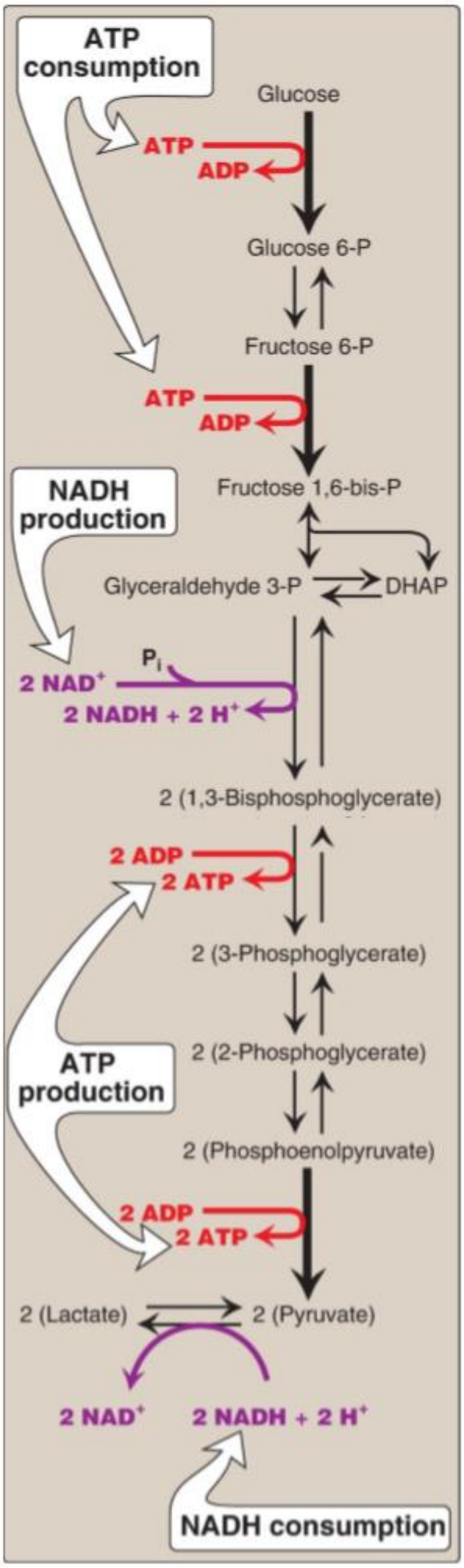

- The enzyme that catalyzes this reaction is **Pyruvate kinase**.
- This reaction is **the third irreversible reaction.**
- **PEP** has a high energy, thus, the Phosphate group has a high ability to **transfer to ADP to form ATP (substrate level phosphorylation)**

- When **PEP** loses its Phosphate group, it will be converted to **enol pyruvate** (OH and double bond at the same carbon ''C2'') which is a highly unstable molecule. Therefore, rearrangement occurs to produce **Pyruvate.**
- In this step **1 (ATP)** is produced **per 1 (PEP)**, So for each Glucose molecule (2PEP) **the net production is 2 ATP.**

❖ **Summary for Glycolysis**

- **Step 1:** consumes 1 ATP molecule
- **Step 3:** consumes 1 ATP molecule
- **Step 6:** Production of 2 NADH
- **Step 7:** Production of 2 ATP molecules
- **Step 10:** Production of 2 ATP molecules

➢ **The net production of Glycolysis is**
   ✓ **2 ATP & 2 NADH**

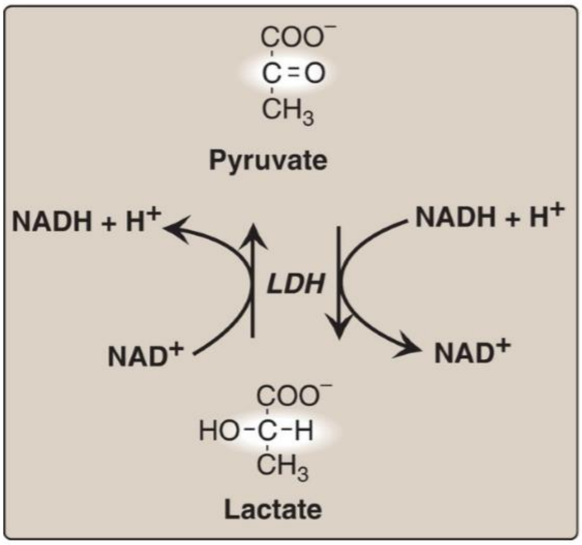



❖ **Is Oxygen Needed?**

- **It depends**
  ✓ **Yes,** if the pathway was **Aerobic Glycolysis,** because **Glucose** concentration is much **higher** than **$NAD^+$** concentration **(limited amount).** Glucose reduces **$NAD^+$** to **NADH**, after a while all **$NAD^+$** will be consumed to form **NADH** which means that this pathway won't go any further. So, to keep the process going **NADH** should be **reoxidized** to **$NAD^+$** and that is done by **ETC** in the presence of **Oxygen**.

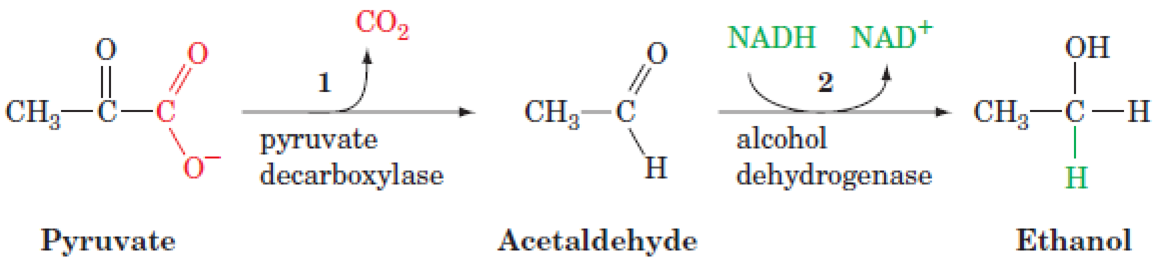
  ✓ **NO,** if the pathway was **Anaerobic Glycolysis**, by reducing the **Pyruvate** (the end product) to **Lactate** (reduction of **ketone** group produces **secondary alcohol**) which leads to the oxidation of **NADH** (reducing agent) to **$NAD^+$**.
  - This process mainly happens in **RBCs** because they don't have mitochondria (NO ETC).
  - The net products of Anaerobic Glycolysis are **2 ATP**, **ZERO NADH**, and **2 Lactate molecules**

## ❖ Pyruvate reduction to lactate

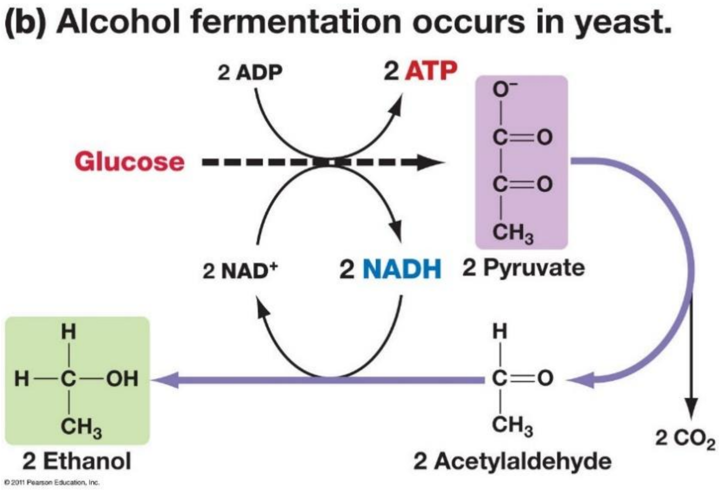



- The enzyme that catalyzes this reaction is **Lactate dehydrogenase (LDH).**
- **LDH** is used for the diagnosis of **Myocardial infarction.**
- **Lactate** is the final product of anaerobic glycolysis in **eukaryotic cells.**
- Lactate is the major fate of pyruvate in **poorly vascularized tissues** and **RBCs.**

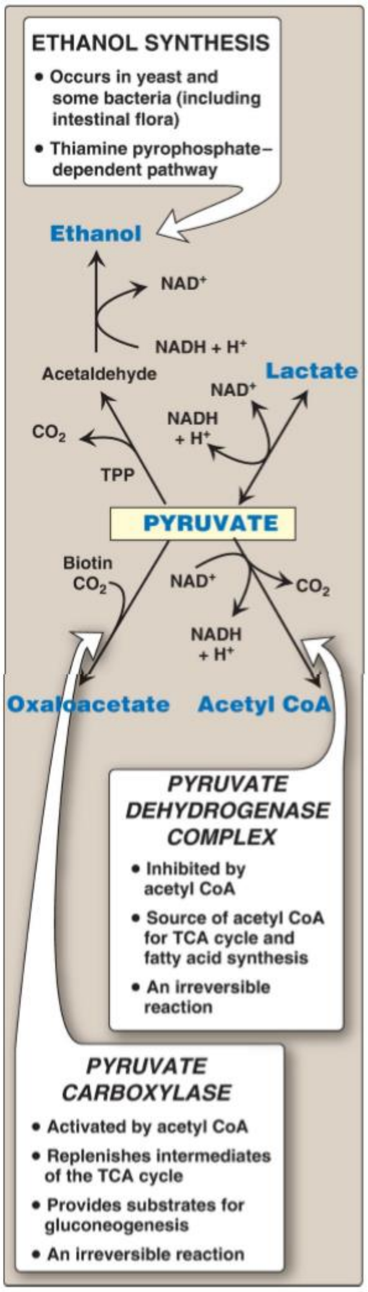
## ❖ Fermentation




- It's another way to reoxidise **NADH** to **NAD⁺**.
- This reaction is catalyzed by **2 enzymes**
  - ✓ **Pyruvate decarboxylase** (converts Pyruvate to Acetaldehyde)
  - ✓ **Alcohol dehydrogenase** (converts Acetaldehyde to Ethanol)
- This reaction occurs in two steps
  - ➢ **First** reaction is catalyzed by Pyruvate decarboxylase in which Pyruvate **is decarboxylated** into Acetaldehyde with the **release of CO2**.
  - ➢ **Second** reaction is catalyzed by Alcohol dehydrogenase in which **NADH** reduces Acetaldehyde to Ethanol (primary alcohol).




- This reaction doesn't take place in humans, but it happens in yeast and some bacteria, and this is why we use yeast in bread making to make dough rise (because of the presence of CO2 ''Gas''). Also, it happens in the making of wine and vinegar.




## ❖ Fate of pyruvate

- **Pyruvate** has several fates, we are already discussed more than one of them
  - ✓ **Pyruvate** to **Acetyl CoA**
  - ✓ **Pyruvate** to **Lactate**
  - ✓ **Pyruvate** to **Ethanol**
  - ✓ **Pyruvate** to **Oxaloacetate** (Note: **Oxaloacetate** can be converted to **Aspartate**)
  - ✓ **Pyruvate to Alanine** (amino acid)
- So, Glycolysis has other routes other than providing energy. It also provides building blocks for making variety of substances.

## ❖ Lactate production

- ➢ **Cells with low energy demand**, like **RBCs**.
- ➢ **To cope increased energy demand** in rigorously exercising muscle, like in a case of running, escaping. Which lead to the increase of the lactate 5 to 10 folds.

- ➢ **Hypoxia** in the case of collapse circulatory system, stop of the heart beat (to survive brief episodes of hypoxia), and that will lead to Lactic Acidosis.

❖ **Lactic Acidosis**

- **Decrease PH in the plasma**, so Lactic Acidosis can be treated by bicarbonate (that will correct the PH in the plasma).
- **The most common cause of metabolic acidosis is**
    - ➢ Increase the production of lactic acid.
    - ➢ Decrease the utilization of lactic acid.

$$\text{Pyruvate} + \text{NADH} \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

- **Most common causes**: impairment of oxidative metabolism due to collapse of circulatory system.

➢ Impaired O2 transport
➢ Respiratory failure
➢ Uncontrolled hemorrhage

- **Direct inhibition of oxidative phosphorylation.**
- **Hypoxia in any tissue.**
- **Alcohol intoxication (high NADH/NAD$^+$),** which will lead to **inhibition** and **the stop of**
    - ➢ Glycolysis
    - ➢ Gluconeogenesis
    - ➢ Pyruvate Dehydrogenase
    - ➢ TCA cycle activity
    - ➢ Pyruvate carboxylase

[Note: the first step in using alcohol as a sores of energy in the body is oxidize alcohol by **Alcohol Dehydrogenase,** that converts **NAD$^+$** to **NADH.**

**Practice Questions**

**Q1:** Whenever the cell's ATP supply is depleted, which of the following enzyme's activity is increased?

a. Hexokinase
b. Pyruvate kinase
c. Glucokinase
**d. Phosphofructokinase-1**

**Q2:** The first step in the payoff phase of glycolysis is

a. Reduction of 1, 3-bisphosphoglycerate to glyceraldehyde 3-phosphate
**b. Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate**
c. Reversible conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate
d. Irreversible conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate

**Q3:** All are characteristic of the conversion of glucose to lactate EXCEPT:

a. anaerobic pathway with no net oxidation.
b. "primed" by ATP phosphorylation.
c. located in the cytosol.
d. approximately 50% efficient in erythrocytes.
**e. net production of four ATP per glucose.**

**Q4:** For the first five steps of glycolysis, the appropriate sequence of enzymes is:
A. phosphofructokinase-1 (PFK-1).
B. hexokinase / glucokinase.
C. fructose bisphosphate aldolase.
D. Phosphoglucoisomerase.
E. triose phosphate isomerase (TPI).

a. A, C, B, E, D
b. B, C, D, E, A
c. B, D, C, A, E
**d. B, D, A, C, E**
e. B, D, E, C, A

**Q5**: All are important reasons to phosphorylate glucose in the first step of glycolysis EXCEPT:

**a. the large positive free energy is important in getting the pathway started.**
b. glucose-6-phosphate has a negative charge preventing transport out of the cell.
c. the concentration of free glucose in the cell is lowered favoring influx of glucose.
d. phosphorylation keeps the glucose in the cell.
e. regulatory control can be imposed only at a reaction not at equilibrium.

**Q6:** The appropriate sequence of steps in the phosphoglucoisomerase catalyzed reaction on glucose-6-phosphate is:
A. creation of a carbonyl group at C-2.
B. opening of the pyranose ring.
C. C-2 proton is removed.
D. furanose ring is formed.
E. formation of the enediol.

a. A, C, E, B, D
**b. B, C, E, A, D**
c. C, E, B, D, A
d. D, E, B, A, C
e. A, C, D, E, B

**Q7:** The step that commits glucose to glycolysis is catalyzed by:

a. hexokinase.
b. phosphoglucoisomerase.
c. **phosphofructokinase-1 (PFK-1).**
d. glucokinase.
e. fructose-1,6-bisphosphate aldolase.

**Q8:** All are characteristics of the phosphofructokinase-1 catalyzed reaction EXCEPT:

**a.** exergonic.
**b.** "priming reaction".
**c.** "valve" controlling the rate of glycolysis.
**d.** commits the cell to metabolize glucose.
**e. all are true.**

**Q9:** Dihydroxyacetone phosphate is:

a. an aldotriose.
b. an enantiomer of glyceraldehyde-3-phosphate
c. derived from C4-C6 of fructose-1,6-bisphosphate.
**d. isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase (TPI).**
e. the least abundant component of the TPI reaction at equilibrium.

**Q10:** The mechanism of triose phosphate isomerase is very similar in function to:

a. hexokinase.
**b. phosphoglucoisomerase.**
c. phosphofructokinase.
d. fructose-1,6-bisphosphate aldolase.
e. none of the above

**Q11:** The first intermediate of glycolysis with free energy of hydrolysis more negative than that of ATP is:

a. glucose-6-phosphate.
b. fructose-6-phosphate.
c. fructose-1,6-bisphosphate.
d. dihydroxyacetone phosphate.
**e. 1,3-bisphosphoglycerate.**

**Q12:** The reaction mechanism for glyceraldehyde-3-phosphate dehydrogenase involves ____ attack by a cysteine -SH group on the ____ carbon of the substrate to form a hemithioacetal.

a. electrophilic; carbonyl
b. electrophilic; acidic
c. nucleophilic; amino
**d. nucleophilic; carbonyl**
e. SN2; amino

**Q13:** A mutase catalyzes which of the reactions, and belongs to which class of enzymes?

a. 2-phosphoglycerate <--> phosphoenolpyruvate + $H_2O$; lyase
b. pyruvate + NADH + $H^+$ <--> lactate + $NAD^+$; oxidoreductase
**c. 3-phosphoglycerate <--> 2-phosphoglycerate; isomerase**
d. dihydroxyacetone phosphate <--> glyceraldehyde-3-phosphate; isomerase
e. glucose-6-phosphate <--> fructose-6-phosphate; isomerase

**Q14:** PEP and 2-PG have similar amounts of potential metabolic energy with respect to decomposition to Pi, $CO_2$ and $H_2O$, but the enolase reaction:

a. creates a much more unstable reactive intermediate.
**b. rearranges 2-PG into a form from which more potential energy can be released by hydrolysis.**
c. rearranges 2-PG to a form with greater binding potential to the enzyme.
d. changes the G of the reaction to increase the potential energy.
e. none are true.

**Q15:** The high free energy change for the conversion of PEP to pyruvate is due largely to the ____ conversion of the relatively unstable ____ tautomer of pyruvate to the more stable ____ form following the phosphoryl group transfer step.

a. unfavorable; enol; keto
b. unfavorable; keto; aldol
c. favorable; keto; enol
d. **favorable; enol; keto**
e. favorable; enol; aldol

**Q16:** Under anaerobic conditions, skeletal muscle generates lactate from pyruvate to:

a. lower the pH.
b. promote release of oxygen from hemoglobin.
c. generate additional ATP.
d. be warning of muscle fatigue.
**e. regenerate $NAD^+$ for further glycolysis**.